REMARKS

The present invention relates in part to methods for staining cells for detection by flow cytometry. In particular, the present claims relate to methods for catalyzing the deposition of tyramide in an analyte-specific manner for the detection of intracellular analytes. The methods of the instant claims can provide at least a 10-fold greater signal than that obtained by standard flow cytometric techniques.

Claims 1-33 are currently pending in the application, with claims 34-61 having been cancelled previously. Claims 1-6, 12, 18, and 23 are amended herein. Support for the amended claims is discussed in the following remarks.

Notwithstanding the foregoing, Applicant expressly reserves the right to pursue subject matter no longer or not yet claimed in the present application in one or more applications that may claim priority hereto. Applicant respectfully requests reconsideration of the claimed invention in view of the foregoing amendments and the following remarks.

Non-Art Related Remarks

35 U.S.C. § 112, second paragraph

Applicant respectfully traverses the rejection of claims 1-33 under 35 U.S.C. § 112, second paragraph, alleging that the claims are indefinite for failing to particularly point out and distinctly claim the present invention. Applicant respectfully disagrees that claims 1-33 are allegedly indefinite in reciting "using isotype/subtype matched nonspecific immunoglobulin as a negative control."

When determining definiteness, the proper standard to be applied is "whether one skilled in the art would understand the bounds of the claim when read in the light of the specification." *Credle v. Bond*, 30 USPQ2d 1911, 1919 (Fed.Cir.1994). See also *Miles Laboratories, Inc. v. Shandon, Inc.*, 27 USPQ2d 1123, 1127 (Fed.Cir.1993) ("If the claims read in the light of the

specification reasonably apprise those skilled in the art of the scope of the invention, § 112 demands no more.").

The examiner is incorrect that "it is unclear what is encompassed by the recitation 'isotype/subtype matched nonspecific immunoglobulin.'" Paper No. 13, page 3. The concept of antibody isotypes and subtypes have long been understood by those of skill in the art. As an example of this understanding, Applicant submits herewith an excerpt from Abbas *et al.*, *Cellular and Molecular Immunology*, 2nd Ed., W.B. Saunders Company, 1994, which indicates that the skilled artisan understands an antibody "isotype" to refer to whether the antibody is IgA, IgD, IgE, IgG, or IgM. (page 39, left column, lines 15-18); and that IgG and IgA are further subdivided into "subtypes" IgA_1, IgA_2, IgG_1, IgG_2, IgG_3, and IgG_4 (page 39, lines 18-20). Applicant respectfully submits that, based on the understanding of this phrase within the relevant art, the skilled artisan is reasonably apprised of what is encompassed by "isotype/subtype matched nonspecific immunoglobulin." *See, e.g.*, MPEP §2173.02 ("Definiteness of claim language must be analyzed, not in a vacuum, but in light of... [t]he teachings of the prior art; and [t]he claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made").

The Examiner is also incorrect that "[i]t is further unclear what structural or functional cooperative relationship exists between the isotype/subtype matched nonspecific immunoglobulin and the other elements of the claim." Paper No. 13, page 3. The claims refer to analysis of cells using flow cytometry. As noted in detail in the specification, flow cytometry relies on the use of an analyte-specific reagent such as an antibody to label cells for quantitation or detection. *See, e.g.*, specification, page 9, lines 1-15. The skilled artisan will readily acknowledge that, while antibodies specifically bind their target antigen (the "intracellular analyte" in the case of the claimed assays), nonspecific protein-protein interactions also result in some level "background" binding. The reference in the claims to a "nonspecific immunoglobulin" used as a "negative control" clearly describes the use of an antibody that does not specifically bind to the cells of interest in order to assess the signal obtained from this background binding.

In the case of the present claims, then, the skilled artisan would readily understand that the "nonspecific immunoglobulin" is an antibody that is not specific for the "intracellular analyte," and that the nonspecific immunoglobulin is "isotype/subtype matched" to the analyte-specific antibody used in the flow cytometric detection of the intracellular analyte. An example of this is shown in Example 1, beginning on page 26 of the specification, where tyramide amplification staining for interleukin 2 is compared to standard flow cytometry (*see* step 4). In this example, the negative control signal is obtained using an IgG_1 isotype/subtype control antibody (*see* step 3).

Thus, when the claims are properly read in the light of the specification, the skilled artisan is reasonably apprised of the scope of the invention. In an effort to advance prosecution, however, Applicant has amended the claims to refer in a more explicit fashion to the interrelation of the various claim elements already described in the claims. As amended herein, the claims indicate that the cells are contacted with an antibody or fragment thereof that is directly or indirectly bound to an enzyme that catalyzes the deposition of tyramide. A signal is detected from the cells, where the signal is at least 10-fold greater than a signal obtainable by standard flow cytometry using an immunoglobulin that does not specifically bind the intracellular analyte, and that is isotype/subtype matched to the antibody or fragment thereof used in the catalyzing step.

Support can be found in the specification for the use of antibodies and antibody fragments, *e.g.*, on page 4, lines 23-25; for direct or indirect binding of the antibody or fragment to the enzyme, *e.g.*, on page 4, line 31, to page 5, line 6; and isotype/subtype controls, *e.g.*, on page 9, lines 30-31.

Applicant respectfully submits that the claims meet the standard of 35 U.S.C. §112, second paragraph, and request that the rejection be reconsidered and withdrawn.

35 U.S.C. § 112, first paragraph

Applicant respectfully traverses the rejection of claims 1-33 under 35 U.S.C. § 112, first paragraph, in which the Examiner contends that the specification does not enable the skilled artisan to practice the claimed invention without undue experimentation.

The standard for determining enablement is whether the specification as filed provides sufficient information as to permit one skilled in the art to make and use the claimed invention. *United States v. Telectronics, Inc.*, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988). The test of enablement is not whether experimentation is necessary, but rather whether any experimentation that is necessary is undue. *Id.* A considerable amount of experimentation is permitted, provided that it is merely routine, or provided that the specification provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

The Examiner purports to analyze each of the factors enunciated in *In re Wands* for determining whether the enablement standard has been met. Applicant agrees that the nature of the invention is directed to methods for detecting the presence of an intracellular analyte using tyramide deposition and flow cytometry. Applicant further agrees that the prior art fails to disclose any such methods in which a signal is at least 10-fold greater than that obtainable by standard flow cytometry methods when isotype/subtype matched controls are performed. Applicant also agrees that the skill in the art is high. Applicant respectfully submits, however, that the Examiner's remaining arguments regarding the various *Wands* factors are set forth entirely without support of objective evidence of record, and are based on a flawed understanding of the concept of antibody isotypes and subtypes.

For example, Applicant disagrees with the Examiner's assertion that "there is no predictability based on the instant specification how the isotype/subtype matched nonspecific immunoglobulin can be used as a negative control." Paper No. 13, page 5. The concept of controls in flow cytometric methods is well established. Moreover, as discussed above, the

concept of antibody isotypes and subtypes have long been understood by those of skill in the art. *See, e.g.*, excerpt from Abbas *et al.*, provided herewith. To perform an appropriate isotype/subtype negative control, the skilled artisan simply replaces a specific antibody with an isotype/subtype matched nonspecific antibody, and performs the method identically in all other respects. Such methods are well established in the art. *See, e.g.*, *Flow Cytometry Protocols*, Jaroszeski and Heller, eds, Humana Press, Totowa, 1998, page 53 ("the use of fluorochrome-labeled control antibodies of the same isotype... is important for flow cytometric analysis of intracellular... marker studies. The control reagents should be used at the same concentration as the specific antibodies"), and page 69 ("Control cells should be treated identically; however, the control cells should be incubated with the same batch of isotypic antibody instead of a [specific] antibody"); Todisco *et al.*, *Blood* 15: 535-542 (2000), page 537, left column (use of isotype-matched "nonreactive antibody" as a negative control in flow cytometry); Cornfield *et al.*, *Arch. Pathol. Lab. Med.* 127: 461-464 (2003), page 462, left column ("Matched isotype controls were used in all FCM panels"); University of Texas continuing medical education program on "*Diagnostic Flow Cytometry*," page 5 of 10 ("The isotype control serves to detect nonspecific binding.... [T]he antibody should always be of the same isotype as the panel antibodies"), each of which is provided herewith for the convenience of the Examiner.

Likewise, Applicant also disagrees with the Examiner's unsupported assertion that "the specification fails to provide adequate guidance to enable use of isotype/subtype matched nonspecific immunoglobulin as a negative control" and that "[t]here are no working examples that exemplify use of isotype/subtype matched nonspecific immunoglobulin as a negative control." Paper No. 13, page 5. As discussed above, to perform an appropriate control, the skilled artisan is well aware that on simply replaces a specific antibody with an isotype/subtype matched nonspecific antibody, and performs the method identically in all other respects. Precisely what further guidance might be required is not specified by the Examiner. As also discussed above, Example 1 provides a working example of precisely such a method in which mouse IgG1 is used

as an isotype/subtype matched nonspecific antibody for mouse IgG1 anti-human interleukin 2. Specification, page 26.

Applicant respectfully submits that the Examiner's statement that the quantity of experimentation necessary would be "undue" (Paper No. 13, page 5) is conclusory and not based upon evidence of record. Moreover, the Examiner's belief that evidence of the unpredictability of the method may somehow be found in "the fact that no prior art has been cited using isotype/subtype matched nonspecific immunoglobulin as a negative control" (Paper No. 13, page 8) confuses novelty and unobviousness with a lack of enablement.

The Examiner supplements the discussion of the various *Wands* factors with additional remarks that are also unsupported by any evidence of record. For example, the Examiner states that "a negative control comprises cells not expressing the antigen, or cells having the intracellular antigen but are stained using an antigen specific antibody in the absence of... the fluorescein." Paper No. 13, page 7. Applicant respectfully disagrees, and requests that the Examiner cite some objective evidence in support of this assertion, particularly in light of the Jaroszeski and Heller, Todisco *et al.*, Cornfield *et al.*, and University of Texas continuing medical education program on "*Diagnostic Flow Cytometry*" publications discussed above, which each discuss the use of nonspecific isotype-matched antibody as a negative control for background signal.

The Examiner's assertion of unpredictability is neither based upon any publication, nor upon any technical argument, but rather is based upon a lack of understanding that isotype/subtype matched antibodies are commonly used by those of skill in the art as negative controls for nonspecific binding in immunologic methods generally, including flow cytometry. In view of the foregoing discussion of the various *Wands* factors, Applicant respectfully submits that one of ordinary skill in the art could readily make and use the claimed invention using the specification as a guide without undue experimentation. Applicant, therefore, requests that the rejection under 35 U.S.C. § 112, first paragraph, be reconsidered and withdrawn.

CONCLUSION

Applicants respectfully submit that the pending claims are in condition for allowance. An early notice to that effect is earnestly solicited. Should any matters remain outstanding, the Examiner is encouraged to contact the undersigned at the telephone number listed below so that they may be resolved without the need for additional action and response thereto.

Respectfully submitted,

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